

## A Synaptic Perspective of Fragile X Syndrome and Autism Spectrum Disorders

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**Keywords:** FMRP, FXS, TSC, ASDs, mTOR, ERK, MNK, Synaptopathies, mGluRs, mRNA metabolism.

## **ABSTRACT**

Altered synaptic structure and function is a major hallmark of Fragile X Syndrome (FXS), Autism Spectrum Disorders (ASDs) and other intellectual disabilities (IDs), which are therefore classified as synaptopathies. FXS and ASDs, while clinically and genetically distinct, share significant comorbidity, suggesting that there may be a common molecular and/or cellular basis, presumably at the synapse. In this article we review brain architecture and synaptic pathways that are dysregulated in FXS and ASDs, including spine architecture, signaling in synaptic plasticity, local protein synthesis, (m)RNA modifications and degradation. mRNA repression is a powerful mechanism for the regulation of synaptic structure and efficacy. We infer that there is no single pathway that explains most of the etiology and discuss new findings and the implications for future work directed at improving our understanding of the pathogenesis of FXS and related ASDs and the design of therapeutic strategies to ameliorate these disorders.

## INTRODUCTION

### ASDs and FXS: Causes and Clinical Features

Approximately 1%–3% of the general population is affected by intellectual disabilities (IDs). IDs are neurodevelopmental disorders defined by significant limitations in intellectual functioning and adaptive behaviors and often exhibit comorbidity with autism (Mefford et al., 2012). ASDs are characterized by high phenotypic heterogeneity, comprising deficits in social interaction and communication, as well as repetitive and stereotyped behaviors (Bhat et al., 2014; Volkmar and McPartland, 2014). ASDs have a strong and complex genetic component with multiple familial inheritance patterns and are estimated to be linked to mutations in as many as 1000 genes (see SFARI database). Over the past decade, genome-wide association studies have enabled rapid progress in the identification of risk genes for ASDs (Ramaswami and Geschwind, 2018).

In addition to the genetic factors, environmental and/or immune factors contribute to the risk of autism during in early life (Waye and Cheng, 2018). The interaction of environmental exposures with an individual genetic background and particular susceptibility manifests differently in each case, leading to heterogeneous phenotypes and varied comorbid symptoms within the disorder. Among the environmental factors to consider are prenatal infections (maternal immune activation), maternal diabetes, zinc deficiency, prenatal and perinatal stress, and toxins (reviewed in Park et al., 2016). Several reports suggest that exposure to various toxicants including pesticides, polychlorinated biphenyls, and polybrominated diphenyl ethers (PBDEs), can have detrimental consequences on developmental processes. The negative effects could be exacerbated in genetically susceptible individuals (Mostafalou and Abdollahi, 2017).

Several genetic syndromes are known to have significant associations with autism (Borrie et al., 2017). Study of these autism-related syndromes, which are well-defined genetic disorders, has yielded major insights into ASDs. For example, Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by the formation of hamartomas in different organs, including the brain, and it is caused by mutations in the *TSC1* or *TSC2* genes encoding hamartin and tuberin respectively. The TSC protein complex inhibits the mechanistic target of rapamycin (mTOR) signalling pathway (Henske et al., 2016; Jeste et al., 2016). Children and adolescents with TSC display epilepsy, cognitive disorders and around 30-40% autism (Curatolo et al., 2010).

Another important example of syndromic autism is Fragile X Syndrome (FXS), the most prevalent neurodevelopmental disorder with a genetic origin characterized by a high incidence of autism. FXS, first described by Martin and Bell in 1943 has a prevalence of approximately 1 in 4000 males and 1 in 8000 females. It is an X-linked condition caused, in the majority of cases, by an unstable CGG trinucleotide repeat expansion (200 or more) in the 5'-untranslated region (UTR) of the fragile X mental retardation 1 (*FMR1*) gene (Bagni et al., 2012), leading to its hypermethylation and transcriptional silencing (Bagni et al., 2012; Berry-Kravis et al., 2018;

Nelson et al., 2013). Over the past years rare *FMR1* gene mutations, affecting the coding or non-coding region of the gene and causing FXS, have also been reported (Sitzmann et al., 2018).

Fragile X mental retardation protein (FMRP), the product of the *FMR1* gene, is an RNA binding protein that tightly controls the localization, stability and translation of a large array of RNAs critical to neuronal development, synaptic plasticity and dendritic spine architecture (for recent review, see Banerjee et al., 2018; Braat and Kooy, 2015; Contractor et al., 2015; Huber et al., 2015; Richter et al., 2015). The behavioral phenotype of children with FXS can vary greatly among individuals, although there are several “core” behaviors seen in the youngest patients. Avoidant eye gaze, Attention Deficit Hyperactivity Disorder (ADHD), hand flapping, repetitive behaviors, reduced social interactions, anxiety and speech perseverations have been reported in 60 to 90% of individuals with FXS (Bagni et al., 2012; Roberts et al., 2018). On the more extreme end, some patients with FXS and autism are completely nonverbal, exhibit aggressive or self-injurious, repetitive and/or stereotyped behaviors, and severe intellectual disability. Around 40% to 60% of male and 20% of female patients with FXS meet the criteria for ASDs (Kaufmann et al., 2017). ASDs seem to be a distinctive subphenotype in FXS characterized by deficits in complex social interaction, with similarities to ASDs in the general population (Hernandez et al., 2009). Individuals with FXS and ASDs also have a lower IQ and more stereotyped and aggressive behaviors, impaired expressive syntax, hyperactivity, emotional lability, attentional deficits, resistance to change, seizures, sleep disorders, hypersensitivity to sensory stimuli and autonomic dysfunction (Bailey et al., 1998; Hatton et al., 1999; Kaufmann et al., 2017; McDuffie et al., 2010; Thurman et al., 2015). Of note, the observed impaired expressive syntax may contribute to atypical social behaviors and thereby, ASD symptoms (Abbeduto et al., 2018).

### **FXS and ASDs: Common features in genetic makeup and brain circuitry.**

Our brains undergo remarkable changes throughout infancy, childhood, and adolescence. Processes including myelination and synaptogenesis (Remer et al., 2017) occur across the first years of life, and brain remodeling continues into young adulthood. Approximately one-third of the human genes are expressed primarily in the brain and during brain development (Douet et al., 2014). Genes that influence developmental and age-specific brain trajectories play an important role in several critical events in neurodevelopmental disorders such as ASDs (reviewed in Parikshak et al., 2015). The identification of large biological networks of genes affected by rare de novo copy number variants in autism revealed that the genes forming networks are primarily related to synapse development, axon targeting and cytoskeleton organization (De Rubeis et al., 2014; Gilman et al., 2011), processes that ultimately shape our brain.

Mutations in many genes linked to ASDs and related disorders affect the excitatory/inhibitory balance in neurons. Perturbations in synaptic physiology and neuronal circuitry have recently been reviewed (e.g., Contractor et al., 2015), and will therefore be

mentioned only briefly here. Hyperexcitability of excitatory neurons is a recurring topic in FXS (see below). In addition, there is evidence for weakened inhibitory drive. Notable examples are decreased GABA<sub>A</sub> receptor expression in the hippocampus of FXS patients and *Fmr1* KO mice (Gantois et al., 2006; Gao et al., 2018; Kang et al., 2017), and a reduction in the frequency and amplitude of miniature and spontaneous IPSCs in the mature amygdala (Olmos-Serrano et al., 2010). Moreover, development of the inhibitory network is delayed. In rodents, GABA<sub>A</sub> receptors switch on at postnatal day ~7 from primarily depolarizing (excitatory) to hyperpolarizing (inhibitory), largely due to a changing Cl<sup>-</sup> gradient (Cherubini et al., 2011). This switch is delayed in layer 4 neurons of the somatosensory cortex of *Fmr1* KO mice (He et al., 2014). Similarly, fast-spiking (FS) interneurons in layer IV of neocortex that lack FMRP remain excitatory throughout the critical period (up to P9, Nomura et al., 2017), with important consequences for synaptic and circuit properties in the mature cortex. Of note, an imbalance in the excitatory to inhibitory ratio (E/I) is a common theme in FXS and other ASDs (Nelson and Valakh, 2015).

### **Brain structure and functional connectivity: lessons from Magnetic Resonance Imaging**

While in the past, studies in brain relied predominately upon gross anatomical measures of brain structure, morphology, and organization, more advanced non-invasive imaging techniques now offer the ability to characterize and quantify a range of functional and microstructural aspects of brain tissue that may be more closely related to fundamental neurodevelopmental processes (Khundrakpam et al., 2016; Lebel and Deoni, 2018). Specifically, MRI produces detailed three-dimensional anatomical images without the use of damaging radiation. In addition, sophisticated MRI imaging techniques yield invaluable information about brain connectivity. Resting-state functional MRI measures brain functional connectivity (FC). Diffusion tensor imaging (DTI) tractography, and especially its most important read-out, the fractional anisotropy (FA), is a measure of axonal integrity. While MRI techniques do not have the spatial and temporal resolution to arrive at the cellular level, they are very powerful because they are *in vivo* and non-invasive, with a high translational potentiality. Over the past years neuroimaging studies have linked genetics with brain development from infancy to young adulthood (Douet et al., 2014), providing complementary information to age-specific brain trajectories based on gene expression.

Neuroimaging studies demonstrate that ASDs and other psychiatric disorders are correlated with diffuse alterations in large-scale functional and structural brain networks (Baribeau and Anagnostou, 2013). Likewise, suboptimal levels of FMRP in the developing brain appear to be detrimental for brain wiring (Dennis and Thompson, 2013). Imaging studies in humans with FXS (Swanson et al., 2018) and ASDs (Ha et al., 2015) reveal aberrant structure of multiple brain regions. Specifically, long-range FC is altered in ASD and FXS patients (Hall et al., 2013; Holsen et al., 2008; Yerys et al., 2017). Additionally, axonal integrity and connectivity as measured by DTI is also affected in patients with FXS who exhibit significantly

larger or smaller grey matter volume in the bilateral caudate nucleus and other regions relative to controls (Sandoval et al., 2018). The white matter in fronto-striatal pathways, as well as in parietal sensory-motor tracts, is also affected (Barnea-Goraly et al., 2003). In addition, microstructural defects as measured by reduction in FA of white matter tracts, predominantly in the corpus callosum, have been found in both FXS individuals and mouse models (Haberl et al., 2015; Swanson et al., 2018).

MRI studies in *Fmr1* KO mice identified reduced volume of certain brain areas (Ellegood et al., 2010). *Ex-vivo* DTI of *FMR1* KO mice revealed a reduced diffusivity in the cortex of juvenile FXS mice suggesting structural changes in axonal fiber patterns and integrity, indicative of subtle changes in connectivity at early developmental stages (La Fata et al., 2014). In addition, *in-vivo* imaging of FXS mice show an anatomical hyperconnectivity phenotype in the primary visual cortex but a disproportional low connectivity with other neocortical regions (Haberl et al., 2015; Zerbi et al., 2018). These observations are consistent with findings in FXS human infants at the onset of the critical period, who exhibit an increased density of DTI-reconstructed fibers relative to that of normal children (Haas et al., 2009; Swanson et al., 2018).

Similarly, deletions and/or duplications in ASD-associated chromosomal regions, such as the 16p11.2 and the 15q11.2, also lead to abnormalities in brain FC and morphology. For instance, FC in the prefrontal cortex has been found to be impaired in humans and mice with the 16p11.2 deletion (Bertero et al., 2018). Patients with deletions and duplications in the 16p11.2 chromosomal region also present global and regional differences in brain structure compared to controls (Blackmon et al., 2018; Martin-Brevet et al., 2018; Qureshi et al., 2014). In the 16p11.2 hemideletion mouse, there are pronounced sex-specific changes in males with increased FA in medial fiber tracts, especially in those proximal to the striatum, which correlate with gene expression patterns associated with neurite outgrowth and the mitogen-activated protein kinase (MAPK) pathway (Kumar et al., 2018). Furthermore, patients with deletions and duplications in the 15q11.2 chromosomal region present abnormalities in white matter architecture and brain structure (Silva et al., 2018; Stefansson et al., 2014; Ulfarsson et al., 2017; Vanlerberghe et al., 2015). Finally, genetic variations in the *CNTNAP2* gene in humans have been associated with ASDs (Peñagarikano and Geschwind, 2012), and KO of this gene in mice leads to reduced FC and aberrant white matter morphology (Liska et al., 2018; Zerbi et al., 2018). In conclusion, there are consistent deficits detected by MRI in ASD patients and the respective mouse models. Of note, there is heterogeneity in the brain regions affected which might be helpful for patient stratification (Ellegood et al., 2010).

## **Spine structure in humans and mice**

The maturation and maintenance of the pre- and postsynaptic compartments depends upon the activation status of a synapse that defines its connection strength (Holtmaat and Svoboda, 2009). It is thought that defects in spines can give rise to impaired synaptic maturation and may contribute to cognitive deficits observed in individuals with this debilitating condition. Of note, more than 50% of the intellectual disability (ID) genes encode for proteins which are part of the pre- or post-synaptic compartments or implicated in synaptic functions by regulating synapse formation, synaptic actin cytoskeleton assembly or disassembly, or synaptic plasticity (Ropers and Hamel, 2005; Verpelli and Sala, 2012) and form the unifying synapse-based theory for cognitive and behavioural deficits.

Human neuropathological and genetic findings on model systems suggest that FXS and ASDs may be conceptualized as diseases of the synapse because of the aberrant dendritic spine morphology and density (Bourgeron, 2015; Zoghbi and Bear, 2012). Golgi staining of postmortem brain of FXS-affected individuals revealed a deficit in the number, structure and morphology of the synapses. Specifically, FXS patients exhibit a higher density of dendritic spines on distal segments of apical and basal dendrites in the cingulate, temporal and visual cortex, with increased incidence of immature spine morphology and a decrease in mature morphologies. Both temporal and visual cortex exhibited significantly longer and fewer short dendritic spines than control subjects (Martínez-Cerdeño, 2017).

Increased spine density has been observed in brain tissues from humans with ASDs, whereas an increase in spine density on apical dendrites of pyramidal neurons in some, but not all, cortical brain areas was detected (Hutsler and Zhang, 2010). Furthermore, reduced developmental spine pruning in layer V pyramidal neurons in postmortem ASD temporal lobe has been shown to correlate with hyperactivated mammalian target of rapamycin (mTOR) and impaired autophagy (Tang et al., 2014). The mouse model for FXS also presents an overabundance of dendritic spines with an immature morphology (reviewed in He and Portera-Cailliau, 2013), even though some studies report no detectable spine alteration in FXS. Thus, stimulated emission depletion microscopy (STED) detected only subtle differences in the *Fmr1* KO mice that are dependent on age and brain region (Wijetunge et al., 2014). It is possible that the apparent discrepancy is due to a difference in technique, region or developmental stages (reviewed in He and Portera-Cailliau, 2013). Mouse models of ASDs with specific genetic mutations such as the *Cytip1*, *Tsc2*, *Shank3* or *Neurologin-1* genes also present abnormalities in spine morphology and/or number (De Rubeis et al., 2013; Oguro-Ando et al., 2015; Pathania et al., 2014; Peca et al., 2011; Schnell et al., 2014; Varghese et al., 2017).

## **Molecular Signaling**

Another aspect of convergence at the synapse is dysregulation of the mTOR and MAPK/extracellular signal-regulated kinase (ERK) signaling cascades. Molecules in these

pathways (Figure 1) such as Tuberous Sclerosis Complex 1 and 2 (TSC1 and TSC2), neurofibromatosis 1 (NF1), Phosphatase and Tensin homolog (PTEN), MAPK-interacting kinase 1 and 2 (MNK1 and MNK2) and ERK1/2 are dysregulated in FXS, TSC1/2, Angelman syndrome, and Rett syndrome (Borrie et al., 2017; Huber et al., 2015; Mellios et al., 2018; Zoghbi and Bear, 2012). Mutations in TSC1/2, PTEN, MNK1 and MNK2 cause an imbalance in protein synthesis. Of note, the mTOR and MAPK pathways were recently found to be dysregulated in blood from patients with idiopathic ASDs and to possibly constitute a molecular signature for the severity of the disease (Rosina et al., 2019, Figure 1). These observations raise the possibility that dysregulation of mTOR and MAPK may be a unifying theme in individuals with FXS and a subset of ASDs (Abrahams and Geschwind, 2008; Subramanian et al., 2015).

Activation of cell surface receptors such as NMDA-type glutamate receptors (NMDARs), metabotropic glutamate receptors (mGluRs) and TRK receptors activate the MAPK via ERK, and the mTOR pathway via the phosphoinositide-3 (PI3K) and AKT kinases. The ERK/MAPK pathway plays an important role in neural progenitor biogenesis, synaptic plasticity and consolidation of memory and its dysregulation is implicated in the pathophysiology of fragile X (Zoghbi and Bear, 2012). ERK/MAPK signaling is hyperactivated in brains of both FXS patients and *Fmr1* KO mice (Sawicka et al., 2016; Wang et al., 2012). Interestingly, the cleavage product of the amyloid precursor protein (APP), the soluble APP $\alpha$ , is upregulated in *Fmr1* KO mice, leading to elevated activation of ERK1/2 (Pasciuto et al., 2015). Furthermore, dysregulated dephosphorylation of ERK in response to mGluR1/5 stimulation was observed in *Fmr1* KO cortical synaptoneurosomes (Kim et al., 2008). Intriguingly, the 16p11.2 locus deletion includes the MAPK3 gene and is associated with ASDs (Fasano and Brambilla, 2011). Mice harboring the 16p11.2 deletion show disruption of the MAPK pathway and behavioral defects. ERK pathway inhibition during development improves the molecular and behavioral defects observed in those mice, suggesting the MAPK pathway as a potential therapeutic approach for 16p11.2 microdeletion syndrome (Pucilowska et al., 2018). Of note, the MAPK and mTOR pathways converge on regulation of protein translation, which plays a crucial role in synaptic plasticity (see below).

mTOR is a central regulator of cell growth, proliferation, cell cycle, ribosome biogenesis, autophagy, translation, and actin polymerization (Costa-Mattioli and Monteggia, 2013; Laplante and Sabatini, 2012). Components of the mTOR cascade are present at synapses where they influence synaptic plasticity *via* regulation of local protein synthesis in dendrites and actin polymerization in spines (Huang et al., 2013; Tang et al., 2002). Whereas mTOR complex 1 (mTORC1) is a master regulator of cap-dependent translation (Darnell and Klann, 2013), mTORC2 is a central regulator of the actin cytoskeleton through its actions on the actin-depolymerizing factor cofilin (Costa-Mattioli and Monteggia, 2013; Laplante and Sabatini, 2012, see also below). Moreover, mTORC2 (but not mTORC1) has been shown to be critical to mGluR-LTD and related behaviors (Zhu et al., 2018).

Fragile X mice exhibit over-activated mTORC1 signaling at hippocampal synapses,



which is causally related to an over-activation of p70 S6 kinase 1 (S6K1, Ronesi et al., 2012; Sharma et al., 2010). Moreover, crossing *Fmr1* KO mice with mice lacking both alleles of the gene encoding S6K1 corrects signaling, spine morphology, and autism-relevant behaviors (Bhattacharya et al., 2016). Although dysregulation of mTORC1 has been documented in Fragile X humans (Hoeffer et al., 2012), mice (Sharma et al., 2010) and flies (Gross et al., 2015), a role for mTORC2 is, as yet unclear. A recent finding that chronic treatment with rapamycin, which inhibits mTOR, had an adverse effects on sleep and social behavior in both control and *Fmr1* KO mice (Saré et al., 2017) may simply reflect the not unexpected observation that not only too much, but also too little, mTOR activity is deleterious (Saré et al., 2017). Interestingly, mTOR dysregulation has also been observed in ASD patients with 15q11-13 duplication, and in a mouse model overexpressing the *Cyfp1*, part of the 15q11-13 region (Oguro-Ando et al., 2015).

### **Lipid signaling at FXS and ASD synapses**

The brain is particularly enriched in lipids and maintains a highly diverse lipid composition. Overwhelming evidence has identified key roles for signaling lipids in mediating membrane traffic and signal transduction at synapses. In particular phosphoinositides (PIPs) have important pre- and postsynaptic functions (Di Paolo and De Camilli, 2006) including the regulation of exo- and endocytosis of synaptic vesicles and postsynaptic receptors, conductance of ion channels, and signaling from activated neuroreceptors such as mGluRs and NMDARs to allow plastic regulation of synaptic function, which is widely believed to underlie cognition and behavior. Furthermore, mechanical deformation of the lipid bilayer is sufficient to modulate the gating properties of several channels (Phillips et al., 2009) suggesting that membrane lipid composition and organization of the spine is different from that of the dendritic shaft, from which these protrusions emerge (Dotti et al., 2014).

In spite of the importance of lipid signaling for regulating synaptic function, its role in synaptopathies remains largely unexplored. Altered lipid signaling is associated with neuronal diseases. Emerging evidence implicates defective PIP metabolism in intellectual disabilities (IDs) and ASDs (Gross, 2017). Loss-of-function mutations in the acid sphingomyelinase (ASM) gene cause Niemann-Pick disease type A (NPA, Schiffmann, 1996), a rare disorder characterized by the accumulation of sphingomyelin (SM) in lysosomes and plasma membrane of neurons, which causes severe ID, premature aging and child death in early age due to the accumulation of sphingomyelin (SM) in lysosomes and plasma membrane of neurons. A contribution to the cause of the disease comes from impaired activity of the plasma membrane calcium ATPase which increases intracellular calcium and oxidative stress (Perez-Canamas et al., 2017).

Therefore, defective lipid signaling seems to be a common theme in the synaptic and neurophysiological deficits underlying ID/ASD and related synaptopathies. In the case of FXS,

a retrospective study suggested that total cholesterol, low density lipoprotein and high density lipoprotein levels are lower in males with FXS than in the general population (Berry-Kravis et al., 2015). In the absence of FMRP, diacylglycerol (DAG) levels in the basal state are increased in the cerebellum of patients with FXS as well as in *Fmr1* KO neurons. At synapses DAG molecules are produced through activation of postsynaptic receptors and DAG signaling triggers dendritic spine growth and stabilization (Kim et al., 2010); excessive DAG would predict the spine properties observed in the *Fmr1* KO neurons (Tabet et al., 2016). Of interest, among the list of candidate FMRP targets there are regulators of the PI3K-based lipid-signaling pathway, mediators of lipid signaling or components that act as downstream effectors of proteins of the PI3K-mTOR-TSC cascade (Ascano et al., 2012; Darnell et al., 2011; Gross et al., 2015; Tabet et al., 2016).

Another important signaling molecule downstream of the PI3K cascade is the glycogen synthase kinase 3 (GSK3), which is inhibited by AKT. This serine/threonine kinase regulates a variety of pathways involved in neurodevelopment, including neurogenesis, migration, axon growth and guidance, and synaptic plasticity (Santos et al., 2014). It is therefore not surprising that dysregulation of GSK3 $\beta$  contributes to a number of diseases such as mood disorders, schizophrenia and Alzheimer disease (King et al., 2014). Some evidence suggests that GSK3 $\beta$  activity is elevated in FXS mice (Min et al., 2009), and modulation of GSK3 $\beta$  activity has proven to be beneficial in FXS, as it reverses the dendritic spine deficits and the level of protein synthesis (Santos et al., 2014). Interestingly, GSK3 $\beta$  activity is down-modulated by lovastatin (Lin et al., 2016), a statin drug used for lowering cholesterol which has also an effect in inhibiting the Ras-ERK1/2 signaling pathway, exaggerated in FXS (Berry-Kravis et al., 2015; Caku et al., 2014; Osterweil et al., 2010). This has set the basis for two pilot open-label trials of lovastatin in humans with FXS were performed and concluded ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). As lovastatin should actually lower cholesterol, low and high density lipoprotein levels, possible problems associated with already excessively low lipid levels in individuals with FXS should be monitored (Berry-Kravis et al., 2015). Although these trials showed potential functional benefit, a placebo-controlled trial is necessary to ascertain lovastatin efficacy in FXS. Clinical trials with statins have shown mixed results in improving cognition and behaviour in individuals with IDs and ASDs as Neurofibromatosis (NF1, van der Vaart et al., 2016), a monogenic form of syndromic autism, although the social and cognitive phenotype in animal models were rescued. Therefore, while the precise mechanisms underlying defective lipid signaling and IDs/ASDs remain to be identified, the availability of FDA-approved drugs targeting lipid signaling might provide a potential avenue for the treatment of IDs/ASDs-related synaptopathies.

### **Metabotropic glutamate receptors**

The Gq-coupled, Group mGluRs are enriched at excitatory synapses throughout the brain, where they are strategically positioned to regulate glutamatergic signaling (Reiner and Levitz, 2018). They are crucial to synaptogenesis and formation of neural circuitry during brain

development (Reiner and Levitz, 2018) and to mGluR-dependent long-term depression (LTD), an activity-dependent, NMDA-independent form of homosynaptic synaptic plasticity at Schaffer collateral to CA1 pyramidal synapses through adulthood (Oliet et al., 1997; Palmer et al., 1997). Importantly, modulation of mGluR5 corrects the bidirectional alteration of synaptic plasticity observed in *Tsc2<sup>+/-</sup>* and *Fmr1* KO mice and the synaptic and cognitive defects were restored in the double mutant model (Auerbach et al., 2011). A recent finding shows that  $\beta$ -arrestin2 provides an important link between mGluR5 and local translation in dendrites (Stoppel et al., 2017).

Expression of mGluR-LTD is mediated by persistent internalization of AMPA receptors and in adolescent mice requires *de novo* protein synthesis (Huber et al., 2002; Waung and Huber, 2009) with a noted requirement for the activity of the immediate early gene *Arc* encoding the Activity Regulated Cytoskeletal Protein that interacts with dynamin and endophilin to facilitate endocytosis of AMPARs (Chowdhury et al., 2006; Shepherd et al., 2006). Notably, *Arc* mRNA is an FMRP target at synapses (Zalfa et al., 2003) and dysregulation of *Arc* activity is implicated in aberrant synaptic plasticity and memory consolidation in *Fmr1* KO mice (Park et al., 2008). Specifically, *Fmr1* KO mice exhibit deficits in Hebbian forms of long term depression and potentiation, altered excitability, and delayed synaptic maturation (Contractor et al., 2015; Huber et al., 2002). *Fmr1* KO mice exhibit exaggerated mGluR-LTD at CA1 synapses leading to elevated protein synthesis, a hallmark of FXS (Huber et al., 2002). Genetic reduction of mGluR5 reverses many phenotypes in *Fmr1* KO mice including exaggerated mGluR-LTD (Dolen et al., 2007).

At synapses, association of mGluR5 with the interacting scaffolding protein Homer is compromised in *Fmr1* KO mice, a feature that is rescued by genetic deletion of a short, dominant negative isoform of Homer, H1a (Guo et al., 2016; Ronesi et al., 2012). The mGluR1/5–Homer complex signals to the PI3 kinase enhancer PIKE, a target of FMRP (Darnell et al., 2011), which engages PI3K/AKT/mTOR signaling in response to synaptic stimulation (Figure 1, Gross et al., 2015). It is now well-established that PIKE is the upstream effector of overactivated mTOR signaling at hippocampal synapse, providing a functional link between loss of FMRP and overactivated mTOR signaling (and critical to mGluR-LTD, Gross et al., 2015; Sharma et al., 2010), FMRP itself has also been shown to be part of a complex with the regulatory subunit of the ubiquitin ligase Cdh1-anaphase-promoting complex (Cdh1-APC) that regulates mGluR-LTD in the brain (Huang et al., 2015).

Given this evidence, a panel of mGluR antagonists has been tested not only in mouse models (MPEP, CTEP, Fenobam, AFQ056, STX107, RO491753), but also in individuals diagnosed with FXS, reviewed in (Bagni et al., 2012; Hagerman et al., 2017; Richter et al., 2015). However, in clinical trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)), these drugs failed to ameliorate the most severe symptoms in humans. Data generated from several independent laboratories using biological models for the disease are in line with a dysregulated mGluR pathway. The possible discrepancy between the results generated in mice and the clinical trials in human

might suggest that patients' stratification together with learning based, outcome measures should be developed (Berry-Kravis et al., 2018)

In addition to Fragile X syndrome, dysregulation of mGluRs have been reported in other forms of intellectual disabilities and ASDs (Mahato et al., 2018). The Phelan-McDermid syndrome (PMS) is a neurodevelopmental disorder caused by genetic haploinsufficiency of the *SHANK3* gene, which is a member of the Shank/ProSAP family of postsynaptic scaffolding proteins (Shank1, Shank2, and Shank3) that regulate multiprotein complex assembly in the postsynaptic density of excitatory synapses, and that has been identified in gene-linkage studies to be associated with autism (Monteiro and Feng, 2017). A mouse model of PMS, the Shank3 KO mouse, exhibits altered mGluR5-mediated signaling due to reduced association of mGluR5 with Homer1b/c and elevated levels of mGluR5 in PSDs, suggesting that mGluR5 function may also be augmented (Wang et al., 2016). Pharmacological manipulation of mGluR5 activity rescued impaired LTD at striatal synapses and elevated neuronal excitability and ameliorated ASD-relevant behaviors (Vicidomini et al., 2017; Wang et al., 2016). Of note, recently a missense variant in the *SHANK3* gene revealed a novel function of Shank3, to recruit Abelson interactor 1 (ABI1) and the WAVE complex to the post-synaptic density, an event critical to synapse and dendritic spine development, a function which is independent of its binding to Homer (Wang et al., 2019). Mutations in the human *SHANK2* gene (also known as ProSAP1) have been associated with ASDs and intellectual disabilities and *SHANK2*-mutant mice carrying a mutation identical to the ASD-associated microdeletion in the human *SHANK2* gene exhibit ASD-like behaviours. Those deficits were ameliorated by a positive allosteric modulator of mGluR5 (Won et al., 2012). Furthermore, overexpression of the scaffolding protein of Homer1a in the basal and lateral amygdala impaired auditory fear conditioning and induced autism-like reduced social interactions (Banerjee et al., 2016). These findings are significant in that mGluRs act through scaffolding proteins and downstream signaling to mediate the correct functioning of synapses.

### **FMRP at synapses**

A recent study has shown that although FMRP is present throughout the brain, its expression varies markedly among different neuronal populations (Zorio et al., 2017). Within a given neuron, FMRP localizes to the soma, developing and mature axons, dendrites and the base of dendritic spines (Antar et al., 2004; Antar et al., 2006; Ferrari et al., 2007; Zimmer et al., 2017). Because neurons are so highly polarized, transport of mRNAs along the axon and dendrites to undergo local, on-site protein synthesis is key for maintaining its proper structural and functional polarity (Bramham, 2008; Cioni et al., 2018; Rangaraju et al., 2017; Tom Dieck et al., 2014). In these subcompartments, FMRP and its homologs, the Fragile X-related proteins (FXR1P and FXR2P) assemble with other RNA binding proteins and coding and noncoding RNAs to form large ribonucleoprotein particles or granules that tightly regulate mRNA transport, translation, stability, and degradation (Akins et al., 2012; Korsak et al., 2016; Pasciuto and

Bagni, 2014a, b). In axons and dendrites, Fragile X granules are transported along microtubules in a translationally-quiescent manner through interactions with kinesin, myosin and dynein motor proteins (Kanai et al., 2004). Different types of Fragile X granules are selectively expressed in specific neuronal circuits where they tightly regulate transport, localization, stability and translation of target RNAs (Akins et al., 2017; Chyung et al., 2018; Korsak et al., 2016).

Several high-throughput approaches such as coimmunoprecipitation followed by microarray analysis (Brown et al., 2001), antibody-positioned-RNA amplification (APRA, Miyashiro et al., 2003) and crosslinking/immunoprecipitation with RNA sequencing (CLIP-Seq, Ascano et al., 2012; Darnell et al., 2011), led to the identification of hundreds of putative FMRP target mRNAs (>1,000 in brain and >6,000 in non-neuronal cells of which ~1000 were validated by RIP-Seq). The effect of FMRP binding to the majority of these target mRNAs still needs to be elucidated. As yet, only a few have been validated at the protein level, i.e., are known to have altered protein expression in the absence of FMRP, and even fewer have been shown to rescue functional *Fmr1* KO phenotypes when their protein abundance is manipulated.

Among the “core targets” of FMRP identified by these and other studies are a large number of mRNAs encoding pre- and postsynaptic proteins including *NMDARs*, *mGluR5*, *PSD-95*, *Shank1-3*, *Homer1*, *PIKE*, *neuroligins*, *neurexins*, *SNAP-25*, *AP-2*, *bassoon*, *synapsin* and calcium channels (Pasciuto and Bagni, 2014b). Among the FMRP mRNA targets, several hundred (precisely 744) are bona fide axonally and/or dendritically localized mRNAs (Figure 2). A substantial number (246) of these mRNAs are also associated with ASDs (Figure 2). A significant part of target mRNAs are transported and translated in an activity-dependent manner (Dictenberg et al., 2008; Ferrari et al., 2007). In dendrites and the postsynaptic compartment, FMRP regulates local ‘on site’ translation of *Arc*,  $\alpha$ -*CaMKII*, *PSD-95*, *Map1b*, *Fmr1* and *GABA<sub>A</sub>* receptors at dendritic spines (Antar et al., 2004; Dictenberg et al., 2008; Zalfa et al., 2007; Zalfa et al., 2003). FMRP also promotes stability of specific mRNAs, such as *PSD-95* and *GABA* receptors, thereby preventing their decay in specific brain regions and in specific subcompartments within a given neuron (Baat and Kooy, 2015; Miyashiro et al., 2003; Zalfa et al., 2007; Zhang et al., 2007).

It is now well accepted that maintaining a fine balance between the synthesis and degradation of RNAs and correct number of mRNAs and RNA-binding proteins is crucial to the proper structure and function of the synapse and, when dysregulated, result in synaptopathies (Grant, 2012) such as FXS and other ASDs (Bagni et al., 2012; Gandal et al., 2018; Klein et al., 2016; Parras et al., 2018). In normal cells, FMRP tightly regulates mRNA stability, transport and translation. Some of these posttranscriptional processes are also affected in other ASDs. FMRP appears to fine-tune the proteome to match the changing needs of the cell/synapse. Neuronal gene expression is controlled with exquisite spatial and temporal resolution, including activity-dependent transcription in the nucleus and local translation at the synapse. Targets of FMRP include many mRNAs that encode synaptic and other plasticity-related proteins, which,

when dysregulated, result in the cognitive impairment and other features in common between FXS and ASDs (Figure 1). Typical of RNA-binding proteins, the targeting specificity of FMRP seems to be determined by a cooperative interplay among several RNA-binding motifs within FMRP itself and/or its binding partners (Achsel and Bagni, 2016).

An additional source of complexity and heterogeneity comes from chemical modification, e.g., methylation and pseudouridylation, of synaptic mRNA and RNA. While enzyme-mediated mRNA modifications are known to regulate cellular mRNA turnover, the role of these modifications in regulating synaptic RNA started to be explored only recently. It was shown that the brain non-coding RNA *BC1* is differentially 2'-O-methylated in the cell body and at synapses and the change in modification affects its interaction with FMRP (Figure 3) in the different subcellular compartments (Lacoux et al., 2012). Methylation of adenosine in RNA to N6-methyladenosine (m<sup>6</sup>A), the most common mRNA modification present in about one quarter of all RNAs (Arguello et al., 2018), appears to be relevant to synaptic pathologies. Recently the synaptic m<sup>6</sup>A epitranscriptome was mapped and shown to be functionally enriched in pathways implicated in neurodevelopmental and neuropsychiatric diseases (Merkurjev et al., 2018).

Additional evidence was generated underlining a key role of FMRP in recognition and regulation of modified RNAs. High methylation levels of FMRP target mRNAs suggest that m<sup>6</sup>A methylation is likely to be used for selective recognition of target mRNAs by FMRP at the synapse (Chang et al., 2017). More specifically, FMRP was bound to the m<sup>6</sup>A sites of its mRNA targets and interacts with m<sup>6</sup>A reader YTHDF2 in an RNA-independent manner. FMRP maintains the stability of its mRNA targets, while YTHDF2 alone promotes the degradation of these mRNAs. These data together suggest that FMRP regulates the stability of its m<sup>6</sup>A-marked mRNA targets through its interaction with YTHDF2 (Zhang et al., 2018). Furthermore, transcriptomic analysis of post-mortem brains of patients with ASDs and FXS revealed hypoediting in the brains of both, which was shared across brain regions and involved many synaptic genes. These findings are mechanistically explained by the interaction of the Fragile X proteins: FMRP and FXR1P interact with RNA-editing enzymes (ADAR proteins) and modulate A-to-I editing (Arguello et al., 2018; Tran et al., 2019).

### **FMRP lost in synaptic translation**

After more than 18 years of studies addressing the properties of neurons lacking FMRP, excessive translation is posited to be a major contributor to the synaptic and cognitive deficits in Fragile X syndrome (Bagni et al., 2012; Banerjee et al., 2018; Darnell and Klann, 2013; Richter et al., 2015). Of note, enhanced mRNA translation was first reported in lymphoblastoid cells derived from humans with FXS (Brown et al., 2001) and at synapses of Fragile X mice (Dolen et al., 2007; Greenough et al., 2001; Zalfa et al., 2003). FMRP represses protein synthesis by different mechanisms regulating initiation (Figure 1) and elongation of mRNA translation (Bagni et al., 2012; Richter et al., 2015; Zukin et al., 2009).

In brain and at synapses several converging lines of evidence suggest that translational repression occurs mainly at the level of initiation (Sossin and Costa-Mattioli, 2018) regulating the assembly of the pre-initiation complex containing the small ribosomal subunits. The efficiency of mRNA translation is regulated through phosphorylation of translation initiation factors such as eIF4E or the eIF4E-binding proteins (4E-BPs). Phosphorylation of eukaryotic initiation factors eIF4E and/or 4EBPs by mTORC1 or MNK1 releases the brake imposed on translation (Bramham et al., 2016), enabling assembly of eIF4E with eIF4G to form the initiation complex. Mice carrying a genetic mutation in eIF4E-BP2 exhibit increased ratio of E/I, increased translation of neuroligins (NLGNs), as well as autistic behaviors. Modulation of *Nlgn1* corrected the defects and worsened autistic phenotypes (Gkogkas et al., 2013; Wang and Doering, 2013). Of note, NLGNs promote synaptic transmission (Varoqueaux et al., 2006) and are regulated by FMRP at synapses (Chmielewska et al., 2018; Darnell et al., 2011; Pasciuto and Bagni, 2014b).

Hypersensitivity to mGluR5 and ERK signaling also contributes to excessive protein synthesis in the hippocampus of *Fmr1* KO mice. At synapses, the MAPK pathway regulates local protein synthesis by phosphorylation of TSC2, thus modulating mTOR activity (Lipton and Sahin, 2014). Moreover, phosphorylation of ribosomal protein S6, primarily a downstream target and effector of mTOR, is also elevated and phosphorylated by ERK *via* the alternative S6 kinase RSK. Finally, MAPK phosphorylates and activates MAP kinase- serine/threonine-protein kinases (MNKs); phosphorylation and activation of eIF4E by MNKs promotes initiation of elongation and translation (Panja et al., 2014).

While the above-mentioned mechanisms regulate general mRNA translation, specific mRNA translation is orchestrated by FMRP via multiple mechanisms (Figures 1 and 3). FMRP regulates translational initiation by binding cytoplasmic FMR1-interacting protein 1 (CYFIP1), which subsequently sequesters and inhibits eukaryotic initiation factor (Napoli et al., 2008). In this complex, CYFIP1 acts as a non-canonical eIF4E-binding protein (4E-BP), which represses translation of target mRNAs by occluding the eIF4G binding site on eIF4E (Napoli et al., 2008). The CYFIP1-FMRP complex represses translation of dendritically localized FMRP target mRNAs such as: *Map1b*, *αCaMKII*, *Arc* and *APP* (Napoli et al., 2008). Under basal conditions, ~30% of neuronal CYFIP1 is contained within the CYFIP1-FMRP-eIF4E complex, and ~70% is contained in the WAVE regulatory complex, consistent with the concept that the same CYFIP1 molecule cannot be part of both complexes at the same time (De Rubeis et al., 2013). Remarkably, synaptic activity in the form of either brain-derived neurotrophic factor (BDNF) or mGluR signaling triggers a conformational change in CYFIP1, from globular (Di Marino et al., 2015) to planar (Chen et al., 2010b). This conformational change is driven in part by the interaction of CYFIP1 with the small Rho GTPase Ras-related C3 botulinum toxin substrate 1 (Rac1) and releases eIF4E from CYFIP1, enabling assembly of eIF4E with eIF4G to form the first translation initiation complex, which can then proceed to position ribosomes on the mRNA (De Rubeis et al., 2013; Napoli et al., 2008). CYFIP1 can be phosphorylated by MNK, releasing translational inhibition and promoting many protein-synthesis forms of synaptic plasticity (Panja

et al., 2014). More specifically, it was shown that at synapses of the dentate gyrus early in translation, MNK triggers release of the CYFIP1-FMRP inhibitory repressor complex from the mRNA. During late-stage translation, MNK regulates the canonical translational repressor 4E-BP2 in a synapse compartment-specific manner. This late stage is coupled to MNK-dependent enhanced dendritic mRNA translation (Panja et al., 2014). Therefore, the FMRP-CYFIP1-eIF4E inhibitory complex ensures an appropriate rate of local protein synthesis in response to synaptic activity, which is crucial for triggering long-term synaptic plasticity at glutamatergic synapses (Figure 1).

Synaptic plasticity requires actin remodeling and protein synthesis; thus, a balance between these two processes is crucial for brain functioning (De Rubeis et al., 2013). CYFIP1 is also a component of the WAVE regulatory complex (a heteropentamer formed by WAVE1/2/3, CYFIP1/2, ABI1/2, NCKAP1 and HPSC300), which promotes actin remodeling through its interaction with the Arp2/3 complex (Chen et al., 2010b; Eden et al., 2002). CYFIP1 shuttles between the FMRP-eIF4E complex and the Rac1-WAVE regulatory complex, thereby linking protein translation to spine dynamics and actin remodeling (De Rubeis et al., 2013). Moreover, treatment of mice with 4EGI-1, an inhibitor of the eIF4E-eIF4G translation initiation complex creates free eIF4E that competes with Rac1 to bind CYFIP1, thus restoring the balance between these signalling pathways ultimately correcting deficits in hippocampal-based memory and spine morphology (Santini et al., 2017). These findings are consistent with the concept that dysregulation of protein translation and actin dynamics are critical to the pathophysiology of Fragile X.

FMRP can repress mRNA translation at the level of initiation through the action of microRNAs such as miR-125a on *PSD-95* mRNA or miR-196a on *HOXB8* mRNA (Li et al., 2014; Muddashetty et al., 2011) or micro RNA 125b on *NR2A* mRNA (Edbauer et al., 2010, Figure 3). In this context the modulation of the translational inhibition occurs through FMRP phosphorylation. Neuronal activity and/or activation of mGluR5 promotes protein phosphatase 2A (PP2A)-dependent dephosphorylation of FMRP, which induces release of the RISC and stimulation of active translation. Activation of mGluRs also promotes ubiquitination of FMRP, which primes FMRP for ubiquitin-based, proteasomal degradation locally (Hou et al., 2006; Nalavadi et al., 2012), likewise releasing FMRP from the complex. In addition to ubiquitinylation, FMRP can be sumoylated with similar effects: FMRP is rapidly sumoylated in response to neuronal activity and by activation of mGluR5 (Khayachi et al., 2018). The increase in sumoylation promotes homomerization and release of FMRP from dendritic RNA granules, enabling local protein synthesis, which promotes spine elimination and maturation (Khayachi et al., 2018). Additional evidence towards a mechanism of FMRP-mediated translational regulation at the level of initiation came from a study showing that the antidiabetic drug metformin rescues some FXS phenotypes in mice, which coincides with selective normalization of ERK signaling and eIF4E phosphorylation (Figure 1, Gantois et al., 2017). Of note, metformin is a drug known to activate autophagy that is also impaired in Fragile X (section below and References), making it a candidate for drug redirection (Tranfaglia et al., 2018).



The role of FMRP, however, is not limited to inhibition of translation initiation. FMRP can also regulate mRNA translation during elongation, via stalled polyribosomes (Darnell et al., 2011), while another study identified FMRP as translational activator for specific mRNAs (Tabet et al., 2016). In the future it will be interesting to address if the identity of the target mRNA determines the regulatory mechanism (Achsell and Bagni, 2016), or if FMRP can switch its mechanistic role depending on the developmental or physiological state of the cell/subcellular compartment.

FMRP regulates the expression and activity of a substantial number of voltage-gated ion channels (Contractor et al., 2015; Ferron, 2016; Richter et al., 2015). For example, FMRP controls the translation of *Kv4.2*, *Kv3.1*, and *HCN1* mRNAs encoding K<sup>+</sup> channels that control neuronal excitability (Brown et al., 2001; Darnell et al., 2011; Miyashiro et al., 2003). In addition to these translation-dependent mechanisms by which FMRP controls channel abundance, FMRP acts *via* non-canonical (translation-independent) mechanisms to regulate activity and stability of other voltage-gated channels by direct protein-protein interactions, which in turn, alters cellular excitability. In particular, the N-terminal region of FMRP contains two Tudor motifs that typically recognize methylated amino acids (Maurer-Stroh et al., 2003) in proteins or FMRP RNA binding partners (Lacoux et al., 2012). FMRP binds and modulates activity/properties of the Slack and BK potassium channels through this domain, while it interacts with calcium channels *via* its carboxy-terminus. The interaction of FMRP with Slack opens the channel (Brown et al., 2010) and in the case of BK channels, which are also expressed presynaptically, causes broadening of the action potential (Deng and Klyachko, 2016). The interaction of FMRP with calcium channels targets the channels for ubiquitin-based, proteasomal degradation and modulates N-type calcium channel density and synaptic vesicular release (Ferron et al., 2014).

## **Synapse elimination and spine pruning**

Synaptic activity influences the number and strength of synapses that form between neurons. During early brain development, synapse formation exceeds elimination, resulting in an excess of immature excitatory synapses. Later, synapse destabilization and elimination reduces the number of synapses, thus refining neural circuits that underlie behavior and cognition (Rakic et al., 1986). Synaptogenesis and synapse elimination follows different time lines in anatomically and functionally distinct regions. Excess synapses are eliminated or pruned in a manner that depends on sensory experience and synaptic activity (Berry and Nedivi, 2017; Chen and Sabatini, 2012; Mostany et al., 2013; Sudhof, 2018).

An important clue as to the mechanism/s by which synapse elimination is regulated during brain development came from studies on the myocyte enhancer factor (MEF) 2 family of transcription factors. MEF2 members are highly expressed in brain and tightly regulated in response to Ca<sup>2+</sup> signaling, and promote expression of a set of genes that restrict the number of synaptic connections in between hippocampal neurons. Three autism genes, namely *Fmr1*, the ubiquitin E3 ligase murine double minute-2 (*Mdm2*), and Protocadherin-10 (*Pcdh10*), a

member of the cadherin superfamily of Ca<sup>2+</sup>-dependent cell adhesion proteins, are required for MEF2-dependent synapse elimination and act downstream of MEF2 (Pfeiffer et al., 2010; Tsai et al., 2012). Upon activation of MEF2, Mdm2 ubiquitinates the synaptic scaffolding protein PSD-95, enabling recognition by PCDH10, which targets PSD-95 to the proteasome for degradation and promotes synapse elimination. FMRP controls expression of eukaryotic elongation factor 1a (EF1a), which in turn prevents MEF2-dependent PSD-95 ubiquitination and synapse elimination (Tsai et al., 2017). Moreover, PCDH10 was discovered to serve as a ligand for the WAVE regulatory complex implicated in control of actin cytoskeletal dynamics, linking PCDH10 to cofilin signaling and structural remodeling of spines (Chen et al., 2014). Of note, human genetic studies have implicated *PCDH10* in ASDs (Morrow et al., 2008) and the MEF2 family member MEF2C as a convergence point for multiple signaling pathways involved in the pathogenesis of ASDs (Gilissen et al., 2014; Tu et al., 2017). These findings reveal novel roles for multiple autism-linked genes in activity-dependent synapse elimination.

### **Actin dynamics at FXS and ASDs synapses**

Synaptic strength is largely influenced by changes in synaptic structure which rely on regulation of receptor signalling, local protein synthesis and structural remodelling of the cytoskeleton (Jedrejewska-Szmek and Blackwell, 2019; Nakahata and Yasuda, 2018). A major player is the actin cytoskeleton, actin being the most abundant cytoskeletal protein in spines (Cingolani and Goda, 2008; Hotulainen and Hoogenraad, 2010; Lamprecht, 2014). One of the key regulators of actin dynamics, and hence synaptic plasticity, is the small Rho GTPase 'Ras-related C3 botulinum toxin substrate 1' (Rac1, Figure 1). First, Rac1-GTP binds to CYFIP1, activating WRC-mediated actin polymerization while at the same time disinhibiting local protein synthesis (see above). Second, Rac1-GTP activates the p21-activated kinases (PAK1-3), which activate LimK. Activated LimK1, in turn, phosphorylates and inactivates its target cofilin. Cofilin is a depolymerizing factor and major determinant of dendritic spine structure that severs the actin filament (F-actin) at its pointed ends (Bamburg and Bernstein, 2010; Mizuno, 2013) to regulate spine morphology, spine motility and synaptic plasticity (Bosch and Hayashi, 2012; Hotulainen and Hoogenraad, 2010; Lin and Webb, 2009; Pontrello and Ethell, 2009). Growing evidence from studies involving non-neuronal cells indicates that mTORC2 regulates the actin cytoskeleton through Rac1 (Hernandez-Negrete et al., 2007; Huang et al., 2013). In addition, cofilin is a direct target of mTORC2 (Tejada-Simon, 2015).

Dysregulation of the synaptic cytoskeleton is a common theme in the pathogenesis of neurodevelopmental disorders and FXS (Bai et al., 2015; Bourgeron, 2015; De Rubeis et al., 2014; Joensuu et al., 2018; Penzes and Cahill, 2012; Penzes et al., 2011; Pyronneau et al., 2017). Considerable evidence indicates that Rac1 signaling is a point of convergence in ASD/IDs. Mutations in Rac1 have been detected in ASD/IDs and disruption of Rac1 signalling contributes to ASD-like behaviours in animal models (De Rubeis et al., 2013; Dolan et al., 2013; Tian et al., 2018; Zeidan-Chulia et al., 2013). Aberrant Rac1 activation leads to either

abnormally weak or strong glutamatergic synapses (Sadybekov et al., 2017). Of note, Rac1 levels are increased in FXS patients (Fatemi et al., 2013) and baseline activation of the Rac1 pathway is upregulated in the *Fmr1* KO mouse model (Santini et al., 2017). Loss of FMRP leads to increased Rac1 signaling, which is mediated by increased interaction between Rac1 and the scaffolding protein CYFIP1. In *Fmr1* KO brains, an increased abundance of the initiation eIF4E-eIF4G and CYFIP1-Rac1 complexes is observed, thereby blunting synaptic plasticity and learning. Treatment of FXS mice with 4EGI-1, which inhibits interactions between eIF4E and eIF4G, restored the equilibrium between the CYFIP1-Rac1 and protein synthesis and thereby reversed defects in hippocampus-dependent memory and spine morphology (Santini et al., 2017).

Formation and maintenance of dendritic spines is maintained through PAK1 and several PAK isoforms have been implicated in IDs (Asrar et al., 2009; Causeret et al., 2009; de la Torre-Ubieta et al., 2010). Abundance and activity of Rac1 and PAK1 are elevated in the somatosensory cortex of Fragile X mice (Bongmba et al., 2011; Castets et al., 2005; Chen et al., 2010a; de Diego-Otero et al., 2009; Dolan et al., 2013) and inhibition of PAK1 rescues, in part, the increased density and length of dendritic spines in *Fmr1* knockout mice (Dolan et al., 2013; Hayashi et al., 2007; Pyronneau et al., 2017). Pak2 haplo-insufficiency results in markedly decreased density of synapses, impaired long-term potentiation, and autism-relevant behaviours in mice due to reduced activity of the key actin regulators LIMK kinase (LIMK)1/cofilin (Wang et al., 2018). PAK1 and PAK3 are also involved in spine morphogenesis and neural plasticity (Boda et al., 2006). Moreover, PAK3 is associated with non-syndromic X-linked mental retardation (Allen et al., 1998; Bienvenu et al., 2000). PAKs are also the molecular targets of SHANK3 linked to autism-related behaviors (Duffney et al., 2015).

There is considerable evidence that implicates dysregulation of LIMK/cofilin signaling in the aberrant spine morphology associated with Fragile X. Cofilin is dysregulated in the somatosensory cortex of young *Fmr1* KO mice and causally related to spine abnormalities (Pyronneau et al., 2017). Constitutively active (non-phosphorylatable) cofilinS3A corrects aberrant spine morphology and density in the somatosensory cortex, indicating a causal relation between elevated cofilin phosphorylation and an overabundance of immature spines. LIMK-cofilin signaling can also be stimulated by bone morphogenetic protein type II receptor (BMPR2). In humans, the full-length isoform of bone morphogenetic protein type II receptor (BMPR2) is elevated and activates LIMK1 (Kashima et al., 2016). Heterozygosity for BMPR2 or pharmacological inhibition of LIMK1 reduced the density of immature spines and restored synaptic function in Fragile X mice. It would be interesting to explore whether BMPR2 is the link between FMRP loss and Rac1 activation or if these represent independent pathways which converge on LIMK1.

## **mTOR and Autophagy**

The correct number of proteins is maintained by a fine balance between protein synthesis and protein degradation. Elevated levels of synaptic proteins can arise as a consequence of elevated translation and/or impaired protein degradation. Autophagy is a process of programmed degradation and recycling of cellular components *via* the lysosomal pathway that is increased during periods of cellular stress (Harris and Rubinsztein, 2011; Mizushima et al., 2008; Nixon, 2013; Wong and Cuervo, 2010). In neurons, mTORC1 is strategically positioned at pre- and postsynaptic sites where it serves as a brake on autophagy. Under nutrient-rich conditions, mTOR phosphorylates Unc-51-like autophagy-activating kinase 1 (ULK-1) at S757 (Figure 4), a target of mTORC1 and well-established anti-autophagy site (Jung et al., 2009). This, in turn, sequesters ULK-1 away from AMP kinase (AMPK) and halts the initiation of autophagy. By contrast, under conditions of starvation, AMPK phosphorylates and activates ULK-1 at S317. Upon activation, ULK-1 promotes phosphorylation and activation of Beclin-1 at S14, a critical step in the ‘nucleation phase’ of autophagy (Russell et al., 2013). Beclin-1 promotes lipidation of LC3-I to generate its lipidated form LC3-II, enabling elongation of the limiting membrane and formation of autophagosomes (Rubinsztein et al., 2012). Upon lipidation, LC3-II localizes to the phagophore membrane, enabling elongation of the limiting membrane and formation of mature LC3-II (+) autophagosomes, which engulf the cargo (Harris and Rubinsztein, 2011; Mizushima et al., 2008; Nixon, 2013). Autophagosomes act *via* cargo adaptor proteins such as p62 to deliver ubiquitinated proteins, protein aggregates and organelles to lysosomes for degradation.

In neurons, autophagy plays an important role in protein homeostasis and is a key regulator of structural remodeling, synaptic plasticity, and memory formation (Nixon, 2013; Shehata et al., 2012). Molecules such as the cargo adaptor protein p62 recognize and bind ubiquitinated proteins and target them for degradation *via* the lysosomal/autophagy pathway. A possible scenario is that in hippocampal neurons from Fragile X mice, overactivated mTOR leads to reduced autophagy and a build-up of a select group of synaptic proteins. It was recently observed that in neurons, inhibition of either ubiquitin-based, proteasomal degradation or the autophagy/lysosomal pathway lead to the buildup of synaptic proteins such as Arc and PSD-95, consistent with the concept that both pathways are involved in degradation of these and potentially other synaptic proteins under physiological conditions (Yan et al., 2018). Neurons from *Fmr1* KO mice exhibited a buildup of ubiquitinated protein aggregates, which colocalized with p62, consistent with the concept that autophagy is impaired in neurons lacking FMRP (Yan et al., 2018). In addition, overactivated mTOR signaling suppresses autophagy in the brains of *Tsc1*<sup>+/-</sup> and *Tsc2*<sup>+/-</sup> mice during postnatal development, and reduced autophagy is causally linked to impaired pruning of spines in cortical layer V pyramidal neurons. The autism-relevant behaviors in the *Tsc* mutant mice suggest that impaired autophagy and its link to impaired spine pruning is likely to be more widespread in at least a subset of ASDs (Tang et al., 2014).

## Conclusions and future perspectives

In this article, we have reviewed dysregulated molecular signaling cascades, receptors, synaptic function and plasticity and spine architecture that underlie the behavioural and cognitive deficits associated with Fragile X syndrome and other forms of syndromic ASDs. We have argued for a partial overlap between the pathogenetic mechanisms that lead to FXS and ASDs. Therefore, advances in our understanding of FXS may also help to inform studies on other ASDs. More importantly, personalized strategies to treat the social endophenotypes of FXS may also be beneficial for other forms of syndromic – and perhaps even idiopathic – ASD.

Several forms of syndromic autism and intellectual disabilities are associated with mutations in genes that regulate protein synthesis and affect structure, transmission and plasticity of synapses (Zoghbi and Bear, 2012). Failure to maintain the precise number of mRNA molecules and RNA binding protein levels are crucial entry points of synaptopathies (Grant, 2012). Neuronal gene expression undergoes changes at very fine spatial and temporal scales, including changes induced by activity-dependent transcription in the nucleus and local translation at the synapse. A fascinating frontier for the study of local translation is the question of heterogeneity in the basic translation machinery at synapses and during development. It was recently shown that the ribosome has a very heterogeneous composition, allowing for selective translation of transcripts responsible for different cellular functions such as metabolism, cell cycle, and development (Shi et al., 2017). If and in which context specialized ribosomes are found at axons and dendrites is the new frontier to brain development in physiological and pathological conditions such as FXS and ASD.

FMRP appears to fine-tune the proteome to match changing needs of the cell/synapse. A multitude of mRNAs that are targeted by FMRP belong to various biological pathways that are affected in ASDs (Figure 1). The targeting specificity of FMRP seems to be determined by a cooperative interplay of several RNA-binding activities (Achsel and Bagni, 2016), e.g., FMRP itself, miRNAs, and/or *BC1* RNA. The full repertoire of FMRP-containing mRNP complexes is, however, far from elucidated, and new experimental and bioinformatic paradigms are needed to gain a thorough understanding of the molecular functions of FMRP. The identification of FMRP synaptic partners - RNA and proteins - their cell specificity and expression during a developmental trajectory will shed light into such a complex neurodevelopmental disorder. The multitude of FMRP direct and indirect effects as well the multitude of genes implicated in ASDs ([www.SFARI.org](http://www.SFARI.org)) are matched by a consistent heterogeneity in the phenotypes of FXS and ASD patients, indicating that the individual genetic and/or epigenetic background determines which FMRP and or ASD gene mechanism becomes prevalent in the expression of the disease phenotype. This calls for disease models that take individual patients' backgrounds into account. The heterogeneity of FXS and ASDs in humans that underlies the individual variability may explain, at least in part, the lack of success of clinical trials aiming solely at decreasing protein synthesis. A recent study highlighted a large variability in the levels of protein synthesis in fibroblasts among patients diagnosed with FXS (Jacquemont et al., 2018). These findings suggest that future clinical trials should aim at stratifying the FXS population, define reliable endophenotypes, and then target them in personalized treatment strategies. For such

stratification, as well as for earlier identification of ASDs, it is first necessary to identify suitable biomarkers, which have to be selected from the existing lists of genetic and molecular biomarkers, as well as from neuroimaging and cognitive studies.

Although research involving the mouse model has informed our understanding of the pathophysiology of Fragile X including deficits in signaling, synaptic plasticity and behavior, and several clinical trials have been implemented based on data from the mouse, there is to date no effective treatment for the cognitive and social interaction deficits observed in humans with FXS. Other disease models, such as the *Fmr1* KO and other ASD rats, have been developed to better mimic the human phenotype (Berzhanskaya et al., 2017; Hamilton et al., 2014; Harony-Nicolas et al., 2017; Saxena et al., 2018). A recent study involving iPS cells derived from patients with Fragile X demonstrated rescue of the synaptic deficits associated with Fragile X by CRISPR-dependent DNA methylation editing (Liu et al., 2018). This and related studies have propelled a paradigm shift from studies involving the Fragile X and ASD rodent models to those involving patient-derived iPS cells (Bhattacharyya and Zhao, 2016; Linda et al., 2018; Sacco et al., 2018). Advantages of iPS cells are several-fold. In particular, iPS cells are derived from fibroblast cells of individual patients and therefore reflect the genomic heterogeneity found in the patient population. iPSC-derived neurons from individuals with FXS have been generated and used to develop a strategy to reestablish FMRP expression by promotor demethylation (Bhattacharyya and Zhao, 2016; Liu et al., 2018). Similar approaches should be used to screen for compounds that reset translational homeostasis, reduce hyperexcitability, and/or accelerate maturation in iPSC-derived neurons from individuals with ASDs and/or FXS. Yet, iPS cells, or even neurons induced from iPS cells, are not a suitable model to study human behavior.

We envision that mechanistic research using animal models and patient-derived stem cells will help researchers to understand the complex etiology of neurodevelopmental disorders such as FXS and ASDs, which will hopefully lead to more effective therapeutical strategies including personalized pharmacogenomics targets.

## **Acknowledgements**

The authors are extremely thankful to Vittoria Mariano and Adrian Lo for their help in preparing the figures and Michael Bennet, Tilmann Achsel, Eleonora Rosina, Nuria Domínguez Iturza, Alexandros Kanellopoulos for critically reading the manuscript and discussions. This work was supported by SNSF, KU Leuven funds (OTF), NCCR Synapsy 51NF40-158776, Italian Fragile X Association, Novartis to CB and National Institutes of Health grant MH-092877, a generous grant from the F.M. Kirby Foundation to RSZ, and a FRAXA fellowship.

We would like to apologies to the colleagues whose work could not be cited due to space limitation.

## Figure legends

**Figure 1. Simplified model of the mTOR – ERK1/2 - WAVE signaling pathways at synapses.** mGluR receptors mediates activation of PI3K via scaffolding proteins such as PIKE and HOMER, leading to the activation of AKT and inhibition of TSC1-TSC2 complex. TSC2 inhibition of RHEB activates mTORC1, resulting in FMRP phosphorylation by S6 Kinase. The phosphorylation status affects its RNA-binding properties as well as its translational regulation. FMRP interacts with the initiation factor eIF4E and regulates translation through the specific eIF4E-binding protein CYFIP1. In *Fmr1* KO mice and FXS individuals PI3K is upregulated, leading to an increased mTOR phosphorylation, culminating in an increased protein synthesis. mTORC2 has a central role in actin remodeling through its actions on RAC1 and LIMK phosphorylation on the actin-depolymerizing factor cofilin (CFL). Proteins encoded by genes associated to ASDs (SFARI database, <http://gene.sfari.org>) are colored in orange.

**Figure 2. Shared genes among FMRP targets, axonal/dendritic transcriptome and ASDs.** Among the 1842 identifies putative FMRP targets, 271 are in common with ASD genes, and 859 are in common with the axonal/dendritic transcriptome. Moreover, 146 genes are shared with all three classes. 1842 FMRP targets are based on the work from (Ascano et al., 2012; Brown et al., 2001; Chen et al., 2003; Darnell et al., 2011; Miyashiro et al., 2003) and the list by (Ascano et al., 2012) filtered for brain expression. 1065 ASD genes are from the SFARI database, AutDB database (Pereanu et al., 2018) and (Mahfouz et al., 2015). 5028 genes from the axonal and dendritic localized transcriptome are based on the work by (Cajigas et al., 2012) and (Gumy et al., 2011).

**Figure 3. FMRP differently recognizes neuronal mRNAs.** Four principal mechanisms of FMRP mRNA target recognition. (A) FMRP can recognize G-quartet structure and G-rich regions of the target mRNA (B) FMRP can binds specific RNA recognition elements (RREs) such as ACUK and WGGA (Ascano et al., 2012) (C). FMRP can bind indirectly the mRNA targets through either the small non-coding RNA brain cytoplasmic RNA 1 (*BC1*) or microRNAs (miRNAs) (D). Modified from (Bagni and Greenough, 2005).

**Figure 4. A schematic of the process and main regulatory machinery of the autophagy.** The metabolic sensors mTOR and AMPK are the main regulators of autophagy activation. mTORC1 phosphorylates ULK-1, inhibiting autophagy. In condition of nutrient depletion and cellular stress AMPK phosphorylates and activates ULK-1 promoting phosphorylation and activation of Beclin-1, a critical step in the nucleation phase of autophagy. Cytoplasmic material is engulfed by double membranes forming a cup-shaped structure called phagophore. Beclin-1 promotes LC3-I lipidation to generate its lipidated form LC3-II, enabling elongation of the

limiting membrane of the phagophore and formation of autophagosomes. Autophagosomes require protein adaptors such as p62 to deliver proteins and organelles to lysosomes for degradation in the autolysosome. FMRP inhibits mTORC1 and *Fmr1* KO mice show an over-activation of mTORC1 with consequent hyperphosphorylation of ULK1/2 and downregulation of autophagy (Yan et al., 2018).



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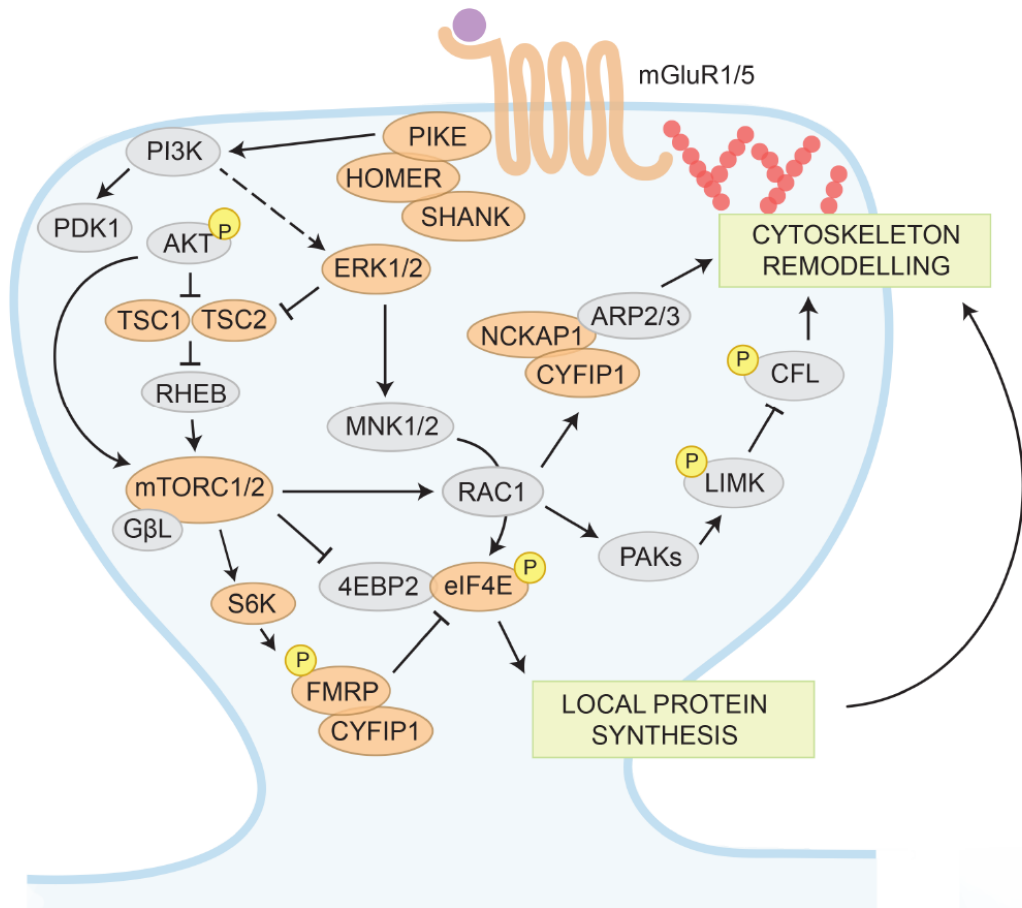
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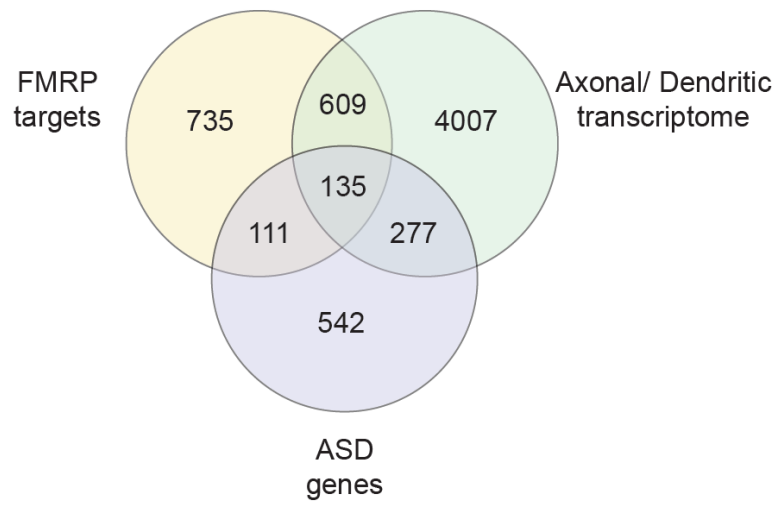
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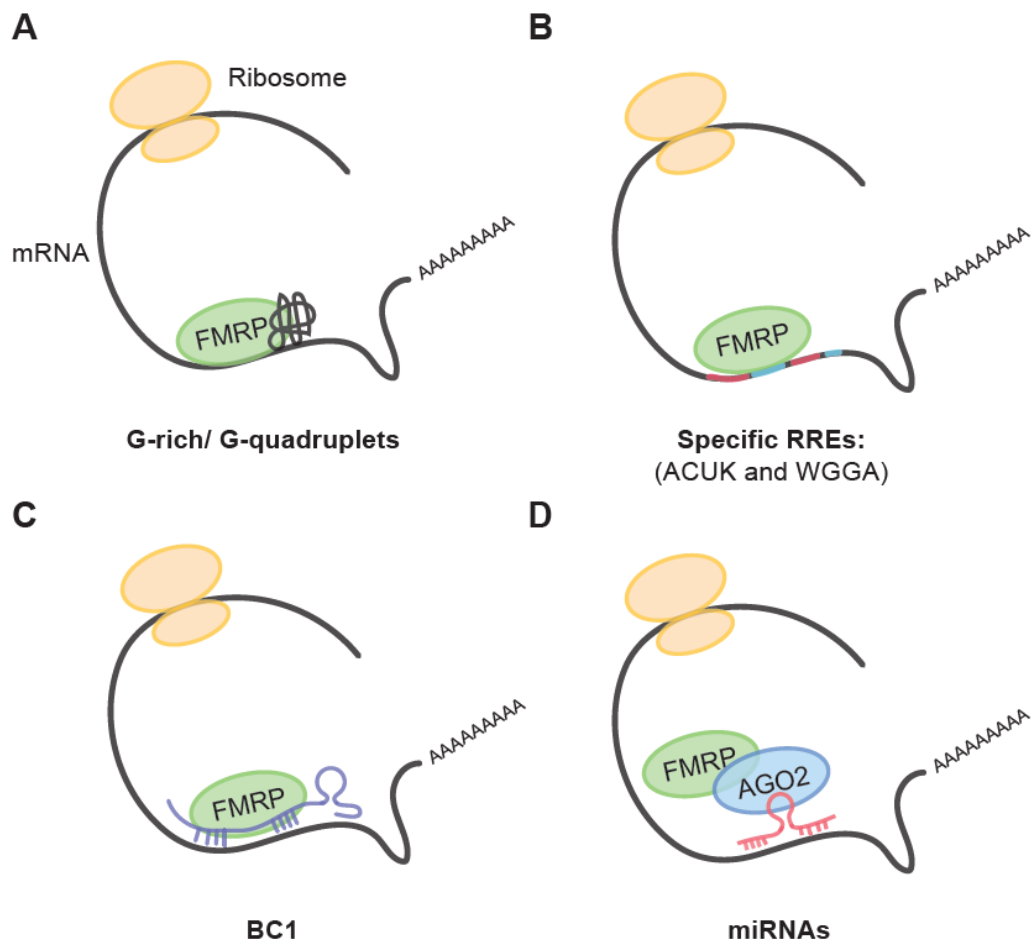
Figure 1



**Figure 2**



**Figure 3**



**Figure 4**

